Successful Validation and Clearance of MALDI-ToF MS for Microorganism Identification

Proteomics in the Clinic Public Workshop

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SCOPE

The comments in this presentation apply only to colony identification using MALDI ToF; they do not pertain to use of mass spectrometry technology for direct specimen testing or nucleic acid amplification methods.

Cleared devices:

http://www.accessdata.fda.gov/cdrh_docs/reviews/K124067.pdf

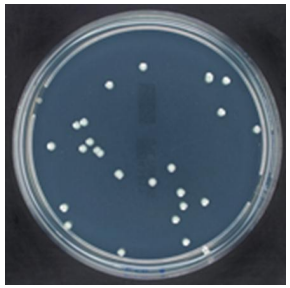
http://www.accessdata.fda.gov/cdrh_docs/reviews/K130831.pdf

MALDI-TOF MS AND COLONY IDENTIFICATION:

Step 1

Sample Preparation

Growth of organism from any specimen source on AGAR media



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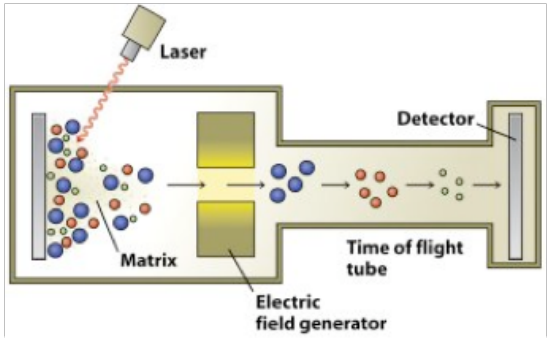
Measurement

- With CHCA matrix
- Automated measurement
- In a specific mass range
- Peak detection



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Results

Pattern matching against the database

MASS SPECTROMETER SYSTEM

- Kit reagents (e.g., matrix)
- Calibrator
- Target slides
- Sample Prep Station
- Reference database
- Software (e.g., acquisition, analysis, middleware)
- Mass spectrometer

REGULATORY – MS INSTRUMENT

§ 862.2860 Mass spectrometer for clinical use. (a) *Identification.* A mass spectrometer for clinical use is a device intended to identify inorganic or organic compounds (e.g., lead, mercury, and drugs) in human specimens by ionizing the compound under investigation and separating the resulting ions by means of an electrical and magnetic field according to their mass.

(b) *Classification.* Class I (general controls). The device is exempt from the premarket notification procedures in subpart E of part 807 of this chapter subject to § 862. 9.

[52 FR 16122, May 1, 1987, as amended at 65 FR 2309, Jan. 14, 2000]

FURTHER...

- Use the mass spectrometer as a diagnostic device, needs to be manufactured under the Quality System Regulations, 21CFR Part 820 and labeled for IVD use Section 809.10.

NOTE: Risk classification comes from the assay, not the instrument

§ 866.3361
Mass spectrometer system for clinical use for the identification of microorganisms

(a) Identification. A mass spectrometer system for clinical use for the identification of microorganisms is a qualitative in vitro diagnostic device intended for the identification of microorganisms cultured from human specimens. The device is comprised of an ionization source, a mass analyzer and a spectral database. The device is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and fungal infections.

(b) Classification. Class II

CORE ELEMENTS FOR PREMARKET SUBMISSION

- Intended use/indications for use
- Pre-analytical Factors
- Analytical Performance
- Clinical Performance
- Statistical analysis plan
- Device description (platform, reagents, software)
- Labeling (package insert)

INTENDED USE FOR MALDI-TOF MS

Type of Test

X is a mass spectrometer system using matrix-assisted laser desorption/ionization - time to flight (MALDI-TOF) for the identification of microorganisms cultured from human specimens.

"Specimen type"

IU Population

X is a qualitative in vitro diagnostic device indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and fungal infections.

How test can be used

The following organisms are claimed: XXXX

Analyte

PRE-ANALYTICAL FACTORS

- Sample preparation
- Sample Stability
- Viability of Sample on Slide
- Culture Media
- Colony Age

SAMPLE PREPARATION

- Direct Transfer to target plate/matrix
- Extraction then transfer to target plate/matrix
  - If organism extraction is recommended, indicate what extraction method and reagents will be used
- Define algorithm of use of each in your study

SAMPLE STABILITY

- Include how long sample material is stable after addition of the matrix.
- Studies performed to determine at what points samples can be stored with appropriate storage conditions prior to and post analysis for sample preparation procedures

VIABILITY

- Within the sample stability study, target spots were cultured to determine viability of organisms (relates to hazard).

CULTURE MEDIA

- Spectral profiles may be influenced by in vitro culture conditions; analytical studies determining the use of these media were conducted prior to the clinical studies.

- Organisms were tested from the media that will be recommended in the package insert.

- If there were differences in the media used to build the database in relation to the media used for the clinical study, analytical studies to determine whether the use of these media affects spectral profiles should be conducted prior to the clinical studies.

COLONY AGE

As age of the colony may affect spectra results, a colony stability study was performed to determine the age range of the clinical specimen isolate that could be tested.

ANALYTICAL STUDIES

- Tolerance study
- Repeatability
- Reproducibility
- Carry over and cross contamination study
- Inclusivity/Exclusivity
- Interference Studies

LOD/ TOLERANCE STUDY

- In terms of colony forming units (CFU)/spot (1 µl), the limit of detection is 10^5 CFU/spot for bacteria and 10^4 CFU/spot for yeast.
- The tolerance study demonstrated that the LoD was different depending of the tested species (e.g., *S.aureus*, *P. aeruginosa*, *E.coli*, *C.jeikeium*, and *C. glabrata*).
- Demonstrated applying an insufficient quantity of colony usually results in no spectra being acquired.
- Demonstrated applying too much colony may cause suboptimal performance of the system.

REPRODUCIBILITY

- Use a panel of 10 coded (blinded) samples with the same controls at 3 testing sites (one of the sites may be an in-house site and at least two sites in the United States).
- Test sources of variability (such as operators, days, assay runs, etc.) for a minimum of 5 days (not necessarily consecutive), with two runs per day, and two replicates of each sample per run.

REPEATABILITY/PRECISION

- Within-laboratory precision studies to include sources of variability (such as operators, days, instruments, assay runs, etc.)
- Include a minimum of 12 days (not necessarily consecutive), with two runs per day, and two replicates of each sample per run.
- Assessed performance between three different instruments, three matrix lots, and three target slide lots.
- The test panel consisted of 10 samples spiked in relevant sample matrix. Assign the first aliquot of the selected samples sequential positions in the run. For subsequent runs, make every effort to randomize the samples in the sequence.
- Reference: CLSI document EP12-A, User Protocol for Evaluation of Qualitative Test Performance, for further information about designing and performing reproducibility and repeatability studies.

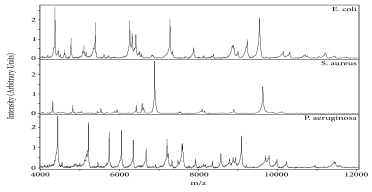
CARRY OVER AND CROSS CONTAMINATION

Alternate a panel of organisms at high positive, medium positive, and matrix alone for low (negative) on the target plate.

A	B		C	A	B	C		A	B	C	A		B	C
B	C		A	B	C	A		B	C	A	B		C	A
		x					x					x		
C	A		B	C	A	B		C	A	B	C		A	B
A	B		C	A	B	C		A	B	C	A		B	C

A = 10^6 CFU/ul
B = 10^3 CFU/ul
C = matrix only
X = E.coli calibrant

INCLUSIVITY/EXCLUSIVITY



- Test other pathogens associated with bacterial or fungal infections that are not claimed by this assay
- Testing multiple strains of bacteria/fungi expected to be identified by the assay.
- Testing closely related organisms within each group

INTERFERENCE STUDIES

Mixed Cultures

- To study mixed cultures, tested claimed organism(s) at the recommended concentration, and non claimed organisms at increasing (low and high) concentrations.
- Indicate how MS instrument report would be issued.

Target Plate Cleaning

- If the target plate is not cleaned and/or rinsed appropriately, determine if interference to sample read will occur.

STABILITY STUDIES

Stability of Target Plates:

- Determine life expectancy of target plates.
- Specify indicators for target plate replacement.

Assay Reagent Stability (e.g., matrix):

- Studies to justify reagent storage and expiration dates. Refer to 21 CFR 809.10.

CLINICAL STUDY PARAMETERS

TESTING AND REFERENCE METHOD

- Large multicenter investigation (5 clinical sites) with good geographic representation using primarily freshly obtained clinical isolates.
- Study relied upon nucleic acid sequence-based identification as the reference method.
- All study isolates were sent to a centralized laboratory for 16s nucleic acid sequence-based identification.
- Discordant Evaluation
- Strict algorithm applied when isolates were tested/retested.

FAMILIARIZATION PERIOD

- Prior to running samples, users became familiar with all the aspects of set-up, operation, maintenance, trouble-shooting, and quality control methods.
- Reference: CLSI guideline EP9-A2, Method *Comparison and Bias Estimation Using Patient Samples.*

BLINDED CHALLENGE PANEL

- Each clinical study site tested a panel of well characterized isolates.

QUALITY CONTROL

- A description of controls used with the device should be provided in the description. (e.g. calibration, external positive and negative controls should be included in each run, external controls should also monitor the organism extraction for each assay run).
- Describe the QC criteria to qualify or disqualify a run and how a run will be validated.
- Run external controls daily throughout the trial (rotating a panel of representative control organisms -reflective of the organisms in the assay menu)

CALIBRATION

- Calibration is an automatic first step in the sample acquisition process; performed with each target slide.
- *A* calibration strain (*Escherichia coli* ATCC 8739) or a bacterial test standard (manufactured extract of E. coli DH5 alpha) was used.
- Each demonstrates a characteristic protein profile mass spectrum when tested with the device.
- Mass range 3 -17kDa

MATCHING ALGORITHM

- **Device 1:** A confidence value is calculated and expresses the similarity between the unknown organism and every organism or organism group in the knowledge These probability results are then provided in the form of a single, species-level identification (green), a split (low discrimination) identification with up to four species-level alternatives displayed (yellow), or no identification (red).

- **Device 2:** The spectrum of unknown organisms is first transformed into a peak list. The peak list is compared to the reference peak list of each organisms found in the database and a log (score) is generated. An organism identification is reported as high confidence if the log(score) is >=2.00, low confidence if the log (score) is between 1.70 and <2.00, and no identification if <1.70.

REFERENCE DATABASE

- The reference database included 5 or more reference strains for each claimed organism.
- For each claimed organism in the reference database, one of the reference strains was a Type strain.
- Each organism in the reference database was well-characterized. Characterization information should be included the submission.
- Database isolates were confirmed with bi-directional sequencing.
- Validated organisms reported; remaining organisms left in database for strength (not reported).

INSTRUMENTATION/SOFTWARE

- Submit all software documentation for the analysis and database software (e.g., acquisition, analysis, middleware)
- Clarify if there is an interface between this software and the laboratory information system (LIS). (e.g. will user be able to export data from the mass spectrometer system and import it into the LIS.)
- Indicate if there is a quality control (QC) software tool to monitor and troubleshoot the MS instrument.
- Describe the algorithm to ensure and monitor the quality of the instrument performance and data generated.
- Provide example reports.

LINE DATA

- Provide electronic data for all analytical and clinical studies.
- Excel files are acceptable, but please refer to the following link which provides explanatory details on presentation format and documentation recommendations: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm136377.htm

STATISTICAL PLAN FOR STUDY RESULTS:

- How study results are reported to sponsor
- How results are analyzed
- Describe statistical tests
- Describe how discordant results are handled
- Definition of true positive, true negative, equivocal, and inconclusive results
- Primary endpoints
- "Statistical Guidance on Reporting Results from Studies
- Evaluating Diagnostic Tests" http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.htm

BENEFIT-RISK ANALYSIS

- Guidance for Industry and Food and Drug Administration Staff - Factors to Consider When Making Benefit-Risk Determinations in Medical Device Premarket Approvals and De Novo Classifications, available at http://www.fda.gov/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm267829.htm.
- It is strongly recommended that your submission include a completed Worksheet similar to that included in the guidance as Appendix B, Worksheet for Benefit-Risk Determinations.

CONCLUSIONS

The basics are the same but one model does not fit all…

- Variety of Specimen Types
- Variety of Pre-analytical steps
- Variety of Analytes (human or pathogen)
- Variety of Mass Spectrophotometry Methodologies
- Different IU = Different Risks
- There are a lot of moving parts……

PRE-SUBMISSION GUIDANCE

Guidance:

Requests for Feedback on Medical Device Submissions: The Pre-Submission Program and Meetings with Food and Drug Administration

http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM311176.pdfdministration Staff

RESOURCES

- Class II Special Controls Guidance Document: *Instrumentation for Clinical Multiplex Test Systems - Guidance for Industry and FDA*, go to: **http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071061.pdf.**
- CLSI M35-A2 – *Abbreviated Identification of Bacteria and Yeast*
- CLSI MM9A Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine
- CLSI MM18AE Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing.
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices
- http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089593.pdf
- FDA Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests *http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071287.pdf*
- Although CLSI C50-A (Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline, Publication Date: 10/29/2007), is not yet recognized by the FDA as a reference standard, it may provide useful supportive information.

THANK YOU!

Questions?

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Thanks to the MS Review Team!

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